MODIFICATION OF FLUOROMETRIC ASSAY FOR THIAMIN IN CHICKEN MUSCLE

by

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TABLE OF CONTENTS

INTRODUCTION 1

REVIEW OF LITERATURE 2

Biological and Chemical Properties of Thiamin 2

Determination of Thiamin 4

Thiochrome method 4

Acid digestion 5

Enzyme hydrolysis 6

Purification 8

Factors Involved in Thiamin Loss 11

The Effect of Cooking Method on Thiamin Retention . . . 13

MATERIALS AND METHODS. 15

Variations in Purification Process 15

Thiamin determination 16

Variables 16

Recovery value 17

Analysis of data 17

Thiamin Content of Fried Chicken Muscles Cooked by Two Methods 18

Preparation of samples 17

Thiamin determination 19

Percentages of moisture and fat 19

RESULTS AND DISCUSSION 19

Variations in Purification Process 20

Eluent flow rate 20

Eluent temperature 22

Eluent volume 24

Thiamin Content of Fried Chicken Muscles Cooked by Two Methods 27

Fat 27

Moisture 27

Thiamin 28

CONCLUSIONS 30

REFERENCES 31

APPENDIX 35

ACKNOWLEDGEMENTS 56

INTRODUCTION

With the increased emphasis on nutritional composition of food products, the need is urgent for rapid, precise and accurate methods of determining nutrients in food. The thiochrome method for thiamin determination has been reported to be rapid, economical, and more applicable to food than most of the other methods (Freed, 1966). Purification of the sample extract by the cation exchanger, Decalso, generally has been recommended if the blanks for sample solutions are high (more than one and half times the blank for the standard thiamin solution). Decalso was the trade name of a resin manufactured by Fisher Scientific Co.; it is no longer available. A properly prepared Decalso column will adsorb thiamin from solution, thereby separating it from interfering substances (Freed, 1966; Pippen and Potter, 1975; AOAC, 1980).

Previous investigators have found that recovery of thiamin from Decalso may be incomplete and irregular indicating that the Decalso purification was a major source of error. Low recovery that is consistent may be acceptable in a quantitative method but with inconsistent recovery, precision and accuracy suffer in proportion to the magnitude of the inconsistency.

Recent studies have reported use of Bio-Rex 70 (Bio-Rad Lab., Richmond, CA), a weakly acidic cation exchange resin in the hydrogen-ion form, in place of Decalso. Bio-Rex 70 was used successfully for the purification of thiamin from a sample extract of animal tissue i.e. raw pork steak, raw pork loin,

and beef (Ellefson et al., 1981 and MacBride and Wyatt, 1983).

Social changes in the last decade, including the increasing involvement of women in the workforce and the growth of ethnic communities, have resulted in growth of fast food restaurants. One of the widely consumed fast foods is fried chicken. Chicken is a good source of B-vitamins. Of all the nutrients present in chicken meat, thiamin is one of the most heat labile vitamins (Lund, 1973). Data on nutritional value of fried chicken cooked by different methods are lacking.

Objectives of this project were : (1) to study the effect that selected variables (temperature, volume and rate of eluent flow using two resins, Decalso and Bio-Rex 70) have on the determined thiamin values of fried chicken meat and (2) to determine the effect of cooking methods on thiamin content of fast-food fried chicken breast muscles.

REVIEW OF LITERATURE

BIOLOGICAL AND CHEMICAL PROPERTIES OF THIAMIN

Thiamin plays a key role as a coenzyme in the intermediary metabolism of α-keto acids and carbohydrates. As a result of this coenzymic role, thiamin can exist in foods in a number of forms, including free thiamin, the pyrophosphoric acid ester (cocarboxylase) and bound to the respective apoenzyme. It has been known as aneurin and the antineuritic factor, indicative

of its role in preventing symptoms involving nerves (Guthrie, 1983). Thiamin, or vitamin B_1, consists of a substituted pyrimidine linked to a substituted thiazole by a methylene group (Fig. 1).

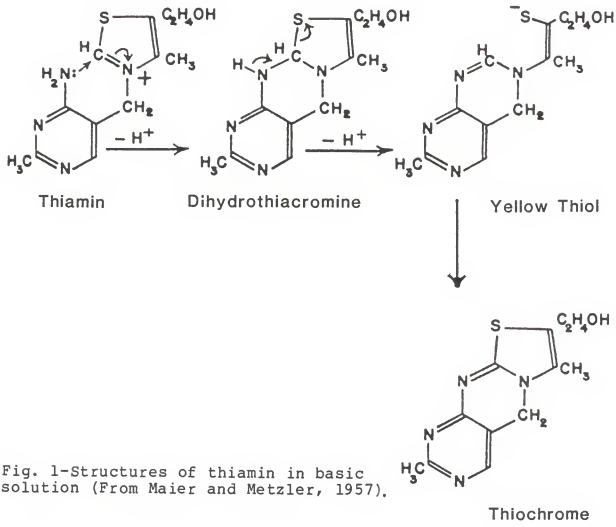


Fig. 1-Structures of thiamin in basic solution (From Maier and Metzler, 1957).

Since thiamin contains a quaternary nitrogen function, it is a strong base and is ionized completely over the entire range of pH normally encountered in foods. In acid solution (at pH 3.5), thiamin will withstand sterilization at temperature of 120°C for one-half hour without loss of activity. In neutral or

alkaline solutions, thiamin is destroyed rapidly, presumably because of decomposition of the thiazole portion. In dry form, the vitamin is very stable and is not sensitive to atmospheric oxidation. In solution, however, thiamin is quite sensitive to oxidation. Mild oxidation under physiologic conditions yields a disulfide of biological activity equal to that of the vitamin (Freed, 1966; Pike and Brown, 1984). When treated with potassium ferricyanide in alkaline solution, thiamin is converted to a fluorescent compound called thiochrome by a reaction involving the removal of two protons. This ease of oxidation has been the basis of the thiochrome method for determining thiamin (Fig. 1).

DETERMINATION OF THIAMIN

Thiochrome method. Chemical analysis is carried out rapidly and economically and is more acceptable to routine determinations than biological methods. Two principal types of chemical determination have been used. One type is a measurement of the color produced by coupling an amine such as p-amino-acetophenone with thiamin (Freed, 1966). The second is the assay of thiamin through its quantitative conversion to thiochrome, which can be determined fluorometrically as proposed by Jansen in 1936. The method is based on the oxidation of thiamin by potassium ferricyanide in an alkaline medium, extraction of the thiochrome formed by isobutanol, and estimation of the intensity of the violet-blue fluorescence in

ultra violet light. The intensity of fluorescence is proportional to the concentration of the thiochrome present, and hence to the amount of thiamin originally in solution.

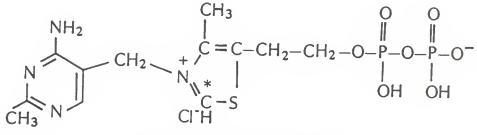
Hennessy (1941) reported that a comparison of the sensitivity of the colorimetric and the fluorometric methods showed that the advantage lies with the latter, particulary in the assay of low potency materials. The amount of thiamin in the aliquot, which was used to produce the pigment in the colorimetric methods, should be more than 5 μg, the preferable range being 20 to 100 μg. For the fluorometric thiochrome procedure, the aliquot needs to contain only 0.1 μg with the preferred range between 0.5 and 2.5 μg of thiamin. Therefore, the thiochrome method has been more applicable to food than the colorimetric methods which could be applied only to relatively pure solutions of thiamin.

Acid digestion. Extraction with hot acid dissolves the various forms of thiamin which may be present. The low pH protects thiamin from rapid destruction during the heating process. Sulfuric acid is preferred to acetic acid for ease and convenience since it does not require refluxing, and for the ease with which it can be adjusted to pH 4.5 - 5.0 by sodium acetate. Heating the sample with sulfuric acid for 60 min resulted in decreased thiamin content in bread indicating that prolonged heat was destructive to thiamin even at a low pH (Bechtel and Hollenbeck, 1958). However, hydrochloric acid was specified as the acid to be used in the digestion because

sulfuric acid may form precipitates that will adsorb thiamin (McRoberts, 1960; Deutsch et al., 1960).

Pelletier and Madere (1975) reported digestion of sample extract by phosphatase for 5 - 48 hr at 40°C preceded or not preceded by acid hydrolysis was suitable for converting thiamin pyrophosphate (TPP) to free thiamin and suggested the omission of acid hydrolysis for both plant and animal products; whereas Edijala (1979) reported that subjecting the sample to both acid and phosphatase hydrolysis significantly increased yields of thiamin in cowpeas.

Enzyme hydrolysis. In many yeasts and animal tissues cocarboxylase, the pyrophosphoric ester of thiamin, is the predominant form of vitamin B_1 while free thiamin (Fig. 1) is the most abundant form in plant materials (Rindi and deGuisepbpe, 1961).



Thiamin Pyrophosphate (TPP)

A phosphatase preparation containing contaminating amylase must hydrolyze the starch present in most food samples, and release thiamin from its phosphate esters. The second function is

vital because thiamin phosphates, although they were converted to thiochrome phosphates, were not extracted by isobutanol and thus were not detected by the assay resulting in inaccurate fluorometric measurement (Hennessy and Cerecedo, 1939; Ellefson, 1981). Hennessy and Cerecedo (1939) succeeded in assaying the total thiamin content of various foods and biological materials by hydrolyzing cocarboxylase to free thiamin with a phosphatase enzyme preparation obtained from beef kidney. No free thiamin was recovered from thiamin pyrophosphate chloride without phosphatase treatment (Edijala, 1979).

Maintenance by a buffer of the pH at the optimum for the phosphatase activity is essential during the entire incubation period if the hydrolysis is to be completed within the alloted time. The optimal pH for the hydrolysis of phosphorylated thiamin by the enzyme present in yeast (diastatic enzymes) is 4.5. The low thiamin value obtained when the reaction was allowed to proceed at pH 8 may be due in part to the instability of the vitamin in alkaline solution (Melnick and Field, 1939; Conner and Straub, 1941).

For the preparation of a large number of samples an incubation period of 20 hr with pH 4.5 at 40^{o}C was found more convenient than the 3 hr period with pH 4.0 - 4.5 at 45 - 50^{o}C recommended by the AOAC (1980) when plant and animal products were incubated (Pelletier and Madere, 1975). However, extending the hydrolysis time from 3 hr to 20 hr for the 2%

enzyme solution did not increase the yield of thiamin in cowpea flour over the pH range 3.5 - 4.8 (Edijala, 1979). An incubation temperature of approximately 45°C was optimal for the hydrolysis of phosphoric ester while at 70°C no hydrolysis was possible owing to the inactivation of the enzyme by heat (Melnick and Field, 1939). A slightly higher temperature of 55°C was used by Ellefson (1981) for incubation of a mixture of 6% enzyme and sample extracts of plant and meat products for 2 hr at 55°C.

From previous studies it appears that the level of enzyme and the incubation temperature and time should be selected properly to release thiamin completely. Foods such as milk are reported to require a proteolytic enzyme treatment in addition to the phosphatase (Halliday and Deuel, 1941). Papain has been recommended for this purpose because its activity at pH 4.5 did not require an incubation separate from phosphatase (Harris and Wang, 1941). However, Hinman et al.(1944) did not obtain higher values of thiamin by the thiochrome technique on papain-clarase or papain-takadiastase digests. Pelletier et al. (1972) also reported that no advantage was gained by simultaneous digestion with papain and clarase agreeing with study of Hinman et al.(1944).

Purification. In a colorimetric or fluorometric assay a purification step is necessary when the solution is colored or when the blank is high, indicating the presence of interfering

materials (Bechtel and Hollenbeck, 1958). A cation exchanger, Decalso, generally has been recommended in the thiochrome method (Freed, 1966; AOAC, 1980). This step consists of passing the impure extract which contains thiamin through the Decalso column. Thiamin is retained on Decalso, from which it can be later removed by treatment of the Decalso column with a potassium chloride solution (Hennessy, 1941).

This process can be a major source of error because small differences in technique, differences in adsorption capacity of the cation exchange material from various sources, and differences in the degree of recovery of the thiamin from the cation exchange column may affect the final result (Kline, 1944). If sample extracts are treated with Decalso, the thiamin standards also should be treated (Freed, 1966; Strohecker and Henning, 1966). Previous investigators have found that recovery of thiamin from Decalso may be incomplete and irregular. Jowett (1940) reported only 68% recovery over a wide range of concentrations. Bechtel and Hollenbeck (1958) obtained recoveries ranging from 84 to 100% and Pippen and Potter (1975) found that recovery of thiamin from Decalso was about 90% and varied $\pm$ 8.5% from the mean recovery value. Harris and Wang (1941) recommended omission of the adsorption step because it caused variable loss of thiamin. Freed (1966) suggested that the 92 - 96% recovery was satisfatory. Important factors affecting thiamin recovery can be eluent temperature, incomplete adsorption of thiamin on Decalso, pH of

sample added to Decalso, decomposition of thiamin during the process, or incomplete elution of thiamin from Decalso (Pippen and Potter, 1975). Recommended flow rate of eluent ranged from less than 1 ml per min (Hennessy, 1941; Bechtel and Hollenbeck, 1958) to 2 ml per min (Edijala, 1979).

Hennessy (1941) and Freed (1966) recommended room temperature of eluent; whereas others have recommended a hot eluent temperature (Pippen and Potter, 1975; AOAC, 1980 and 1984). However, boiling acid potassium chloride should be avoided because it may cause clogging of the Decalso columns as the potassium chloride crystallizes from the supersaturated solution, with a resultant loss of the sample (Freed, 1966). Eluent temperature definitely affected recovery of thiamin from Decalso with hot eluent (90 - 95^{o}C) recovering about 11% more thiamin than cold eluent when recoveries of thiamin were determined by comparing the concentration of purified standard with the concentration of the standard which had not been passed through the Decalso columns. However, Pippen and Potter (1975) recovered only about 87 - 90% with hot eluent. Increases in temperature, which tend to weaken ionic attraction to the resin, also can be used to decrease elution times. Recommended eluent volumes ranged from 10 ml (Hennessy and Cerecedo, 1939) and 15 ml (Bechtel and Hollenbeck, 1958) to as much as 50 ml (Strohecker and Henning, 1966; Pippen and Potter, 1975); but 25 ml was recommended most commonly (Freed, 1966; AOAC, 1980). Increasing eluent volume from 25 to 50 ml

significantly increased thiamin recovery (mean recovery increased from 90 to 97.5%) and decreased the standard deviation from 2.15 to 1.53 (Pippen and Potter, 1975). Hence, eluent volume was an important factor affecting both the quantity and reproducibility of thiamin recovery from Decalso.

Ellefson (1981) recommended using Bio-Rex 70 (hydrogen form) in place of Decalso in the official AOAC method because of the unavailability of Decalso. The recovery of a thiamin standard from Bio-Rex 70 (hydrogen form) was 95 - 100%; whereas the recovery of thiamin from Decalso was 90 - 100%. The low recoveries of thiamin (38%) from Bio-Rex 70 (sodium form) was attributable to the instability of thiamin to the alkaline pH (10.8).

FACTORS INVOLVED IN THIAMIN LOSS

Temperature, pH and time of heating, processing or storage are the most important factors contributing to the loss of thiamin in food products (Dwivedi and Arnold, 1973). Thiamin is degraded readily in neutral and alkaline solutions even at low temperatures (Borenstein, 1975). Feaster et al. (1947) subjected buffered thiamin solutions to heat at temperatures in the range of 180 to 250°F. They concluded that: a) an increase in pH value from 3.5 to 7.0 caused a progressive decrease in thiamin retention; b) with a fixed pH value and heating time, thiamin retention decreased with increasing temperature; and c) at a fixed pH value and temperature,

thiamin losses were approximately proportional to time of exposure. Another study done by Feliciotti and Esselen (1957) showed that the rate of thiamin destruction in phosphate - buffered solutions in the pH range of 4.5 to 7.0 increased with increasing pH. The most pronounced change in the reaction rate occured between pH 6.0 and 6.5. They also found that the variation in thiamin destruction rates was dependent on an interrelationship between hydrogen ion concentration and free thiamin content. Products having a low relative hydrogen ion concentration were able to counteract the high relative proportion of combined thiamin, which would tend to increase the destruction rate. In the case of meat products, the variation in hydrogen ion concentration was less than that of vegetables. Therefore, the difference in thiamin destruction rates may be directly associated with the concentration of combined thiamin. According to Rice and Beuk (1945), at temperature above 77°C, the rate of loss of thiamin was consistent at any given temperature and was proportional to the temperature. Below 77°C, the rate of loss decreased during the first 16 to 24 hr, and then apparently remained constant. In this lower temperature range, the rate of loss was proportional to the temperature.

Additional factors, besides pH and heat, which contribute to thiamin degradation are oxidation-reduction systems, inorganic bases, enzymes, metal complexes and radiation (Dwivedi and Arnold, 1973).

THE EFFECT OF COOKING METHOD ON THIAMIN RETENTION

Because meat is one of the best dietary sources of thiamin, it is desirable to retain thiamin in meat during preparation. Lund (1973) noted that thiamin is one of the most heat labile vitamins. Therefore, cooking destroyed a larger percentage of thiamin. Thiamin retentions in meat range from 90% (wet-weight basis) in fried beef (Tucker et al., 1946) down to 29% (moisture-fat-free basis) in pressure-cooked lamb stews (Cover and Dilsaver, 1947).

The stability of thiamin in meats during cooking depended partly upon the method of cooking. A long cooking process resulted in more destruction of thiamin ranging from 78% in underdone pork chops to 48.8% in the overdone (Jackson et al., 1945). Tucker et al. (1946) reported thiamin retention in beef cooked by different methods : braising, 61% ; broiling, 80% ; and frying, 90% . Hall and Lin (1981) obtained greater thiamin retention in broiler meat cooked to an internal temperature of 92°C in a microwave oven than when cooked in an electric oven to an internal temperature of 82°C indicating that the shorter cooking period more than offset the effect of the higher endpoint temperature. Broiler meat retained significantly more thiamin when cooked at 204°C for a shorter time than when cooked at 121°C for a longer time.

Ang et al.(1978) reported that thiamin retention was lowest in chicken parts reheated by convection and held hot for 3 hr

(74%). Infrared reheating resulted in 84% thiamin retention, while microwave reheating retained 92%. However, Bowers and Fryer (1972) reported similar thiamin values for turkey cooked in gas and in microwave ovens. Therefore, no consistent trend has been observed in thiamin retention in microwave heated muscle.

Generally, more thiamin is retained if cooked by dry rather than by moist methods because thiamin may be leached during cooking or destroyed by high cooking temperature. Mickelsen et al. (1939) found that frying produced the least change in thiamin with the almost complete preservation of thiamin in fried beef round and fried pork ham, while with roasting, broiling or stewing, the destruction of thiamin approached 50%. A problem associated with stewing was that cooking in water caused losses of the water soluble vitamin due to leaching. Low retention of thiamin in roasts was due to the high temperature and extended cooking times often used. Pressure cooking of chicken meat significantly reduced thiamin retention to 38%. However, reduced cooking time could negate this loss. Pressure cooking is a fast method to cook and tenderize chicken; however, large amounts of thiamin can be destroyed due to the high temperature (Pudelkewicz et al., 1963). Gilbert et al. (1981) observed thiamin contents of spent hens' breast muscles cooked by roasting in an oven at 135°C for 5 hr, boiling in water for 2 hr or cooking under pressure for 30 min at 10 lbs pressure were not significantly

different, probably because they used more severe cooking methods than other workers.

Breading may provide some protection against the loss of thiamin caused by leaching and the destruction of thiamin as a result of oxidation. The use of a breading greatly reduced cooking loss and functioned as a moisture barrier regardless of the cooking methods (Love and Goodwin, 1974; Proctor and Cunningham, 1983). Morgan (1971) found coatings excluded oxygen from the food product. Lachance et al. (1973) investigated the effects of reheating convenience foods and found that loss of thiamin in covered samples was around 7% and in uncovered samples was around 20% indicating that covering food products protects them from thiamin loss during heating ; uncovering may be exposing the thiamin to more rapid oxidation.

MATERIALS AND METHODS

Selected methodology variables in the purification step for thiamin determination for fried chicken meat were studied. The thiamin content of fast-food fried chicken muscle cooked by two methods was determined.

Variations in Purification Process

Duplicate determinations of thiamin content of 4 chicken samples were made. Frozen, deboned and homogenized samples

of fried chicken, including coating, were from a fast-food fried chicken chain.

Thiamin determination. Thiamin analysis was by a modified AOAC (1980) thiochrome method. The assay procedure consisted of a 4-step operation involving (1) the extraction of the thiamin from the sample; (2) purification of the extracted thiamin through a cation exchange resin; (3) the oxidation of thiamin to thiochrome by potassium ferricyanide in an alkaline medium, extracting the thiochrome formed with isobutanol; and (4) the quantitative determination of the thiochrome so formed by measuring the intensity of the violet-blue fluorescence with a Coleman model 12C fluorometer. Two readings were averaged for duplicate determinations of thiamin content.

Duplicate sample extract and standard thiamin (thiamin hydrochloride obtained from Sigma Chemical Company, PO Box 14508, ST Louis, MO 63178) solutions simultaneously were passed through the Decalso and Bio-Rex 70 (Bio-Rad Lab., Richmond, CA) columns for each of 4 replications. The specific procedures of the modified AOAC thiamin assay are detailed in Appendix 1.

Variables. Three experiments were designed to study flow rate, eluent temperature and eluent volume using 2 different resin: (1)8 treatment combinations for 4 eluent flow rates (1/2, 1, 1 1/2 and 2 ml per min) and 2 cation exchangers (Bio-Rex 70 and Decalso); flow rate was controlled approximately by

attaching polyethylene tubes (2.5 mm o.d. x 9 cm long) with plastic clamps to the tip of capillary; (2)4 treatment combinations for 2 eluent temperatures (hot and room) and 2 cation exchangers (Bio-Rex 70 and Decalso); for hot elution, acid KCl reagent was heated to 90 - 95°C on the Corning Hot Plate (Model PC-101); for cold elution, reagent was at room temperature; and (3)4 treatment combinations for 2 eluent volumes (25 and 50 ml) and 2 cation exchangers (Bio-Rex 70 and Decalso).

Recovery value. The percentage recovery of standard thiamin was determined by dividing the concentration of purified standard by the concentration of the standard without using the column and multiplying by 100.

Analysis of data. Data were subjected to analysis of variance using the following designs.

Flow Rate:

Source of variation	Degrees of Freedom
Replication	3
Resin	1
Rate	3
Resin x Rate	3
Error	21
Total	31

Eluent Temperature:

Source of variation	Degrees of Freedom
Replication	3
Resin	1
Temperature	1
Resin x Temp.	1
Error	9
Total	15

Eluent Volume:

Source of variation	Degrees of Freedom
Replication	3
Resin	1
Volume	1
Resin x Volume	1
Error	9
Total	15

When F-values were significant, least significant differences (LSD) at the 5 % level were calculated.

Thiamin Content of Fried Chicken Muscles Cooked by Two Methods

Preparation of samples. At each of 4 times, two "Original" and two "Crispy" fried chicken breast portions were purchased at a local fast-food fried chicken chain. "Original" was pressure fried and "Crispy" was open-vat fried. The coating was removed from the chicken pieces. Samples were skinned, deboned and homogenized with a Sunbean Food Processor for 1

min.

Thiamin determination. Thiamin analysis was done by a modified AOAC method (1980). The sample extracts were purified by Bio-Rex 70 columns. A flow rate of 1 1/2 ml per min and 25 ml of eluent at room temperature were used. The thiamin content of fried chicken meat was calculated on a wet weight basis and also on a moisture and fat-free basis.

Percentages of moisture and fat. Percentage moisture was determined by placing 1 g chicken meat in a preweighed aluminum pan and drying overnight in an oven (Franklin Products Corp. Model No. PW-1) at 105°C. Percentage fat was determined by a modification of the Folch method (Folch et al., 1957), substituting methylene chloride for chloroform and extracting the residue twice. The specific procedure of the modified Folch method is detailed in Appendix 2.

Data were subjected to analysis of variance to determine if there were significant differences between thiamin contents and percentages of fat and moisture of "Original" and "Crispy" fried chicken meat.

RESULTS AND DISCUSSION

The effects of selected methodology variables in the purification step for thiamin determination of fried chicken meat and thiamin content of fried chicken muscle cooked by two methods were evaluated. Data for all replications are

presented in Tables 5-15, Appendix.

Variations in Purification Process

Eluent flow rate. Instrument readings of fluorescence, thiamin content of chicken meat, recovery (%) from columns of thiamin from a standard solution at various flow rates are presented in Table 1. Flow rate had less effect on values when Bio-Rex 70 was used than when Decalso was used. For solutions passed through Bio-Rex 70 columns, instrument readings of fluorescence of standard solutions, values for thiamin content of chicken meat, and percentage recovery of thiamin standard were not influenced by flow rate. A recovery value of about 97% was obtained at all flow rates. Fluorescence readings of samples were significantly reduced only when the rapid flow rate was used.

When Decalso columns were used, slower flow rates resulted in greater fluorescence readings for the thiamin standard ($p < 0.05$) and the sample extract ($p < 0.001$) and a greater percentage recovery of thiamin standard ($p < 0.05$). At slow flow rates of 1/2 and 1 ml per min, thiamin recoveries were about 94 and 97% which may be satisfactory according to Freed (1966). But at flow rates of 1 1/2 and 2 ml per min, recoveries were very low (around 82%). Fluorescence readings of solutions passed through Decalso columns were less precise and standard deviations of thiamin recovery values were greater than those passed through Bio-Rex 70 columns. Eluent flow rate was an

Table 1-Instrument readings of fluorescence for thiamin standard and sample extract from ion-exchange columns, thiamin content of fried chicken breast muscle and recovery of thiamin standard at various flow rates[1]

Flow Rate (ml/min)	Fluorescence: Standard	Fluorescence: Sample	Thiamin (mg/100g)	Recovery[2] (%)
Bio-Rex				
1/2	38.4[a]	16.4[a]	0.085	97.1±3.1[a]
1	38.5[a]	15.8[a]	0.082	97.2±3.3[a]
1 1/2	38.7[a]	15.5[ab]	0.080	97.7±0.8[a]
2	38.5[a]	14.2[bc]	0.075	97.3±4.5[a]
Decalso				
1/2	38.2[a]	15.8[a]	0.082	96.5±1.3[a]
1	37.1[a]	15.8[a]	0.086	93.9±10.8[a]
1 1/2	32.6[b]	14.0[c]	0.085	82.5±3.8[b]
2	32.7[b]	13.5[c]	0.084	82.4±8.3[b]
Significance of F-value[3]				
Resin	***	ns	ns	***
Rate	*	***	ns	*
Rate X Resin	*	ns	ns	*
LSD[4]	3.06	1.44	-	7.66

[1] Means of 4 replications

[2] Based on fluorescence of the thiamin standard before adsorption on ion-exchangers

[3] * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level; ns not significant

[4] LSD, least significant difference at the 5% level

[a,b,c] Means with the same letter indicate no significant difference between those means

important factor affecting thiamin recovery when Decalso was used, probably because the rates of ion exchange in zeolite, Decalso, are low compared to those for the organic resin, Bio-Rex 70, owing to the absence of swelling of Decalso. Decalso consists of a synthetic alumino silicate gel which is inorganic (Hampel and Hawley, 1973). Bio-Rex 70 is a weakly acidic cation exchange resin which contains carboxylic acid exchange groups on a macroreticular acrylic polymer lattice (Bio-Rad Lab., 1982).

Eluent temperature. Results of the use of two different eluent temperatures on two different cation exchangers for the purification step are reported in Table 2. For solutions passed through Bio-Rex 70 columns, fluorescence of thiamin standard, thiamin content of chicken meat and recovery (%) of thiamin standard were not affected by increasing eluent temperature. With hot eluent the standard deviation of thiamin recovery decreased from 2.7 to 0.6. Fluorescence readings for sample extracts eluted with hot solutions of KCl, were greater than those with room temperature solutions when using both types of columns. Although eluent temperature did not affect fluorescence readings of thiamin standards or recovery values when Bio-Rex 70 was used, a hot eluent temperature increased fluorescence readings and percentage recovery for standard solutions purified by Decalso. Values for thiamin content were not affected, because the calculation

Table 2-Instrument readings of fluorescence for thiamin standard and sample extract from ion exchange columns, thiamin content of fried chicken breast muscle and recovery of thiamin standard at room and hot temperature (90-95°C)[1]

Temperature	Fluorescence: Standard	Fluorescence: Sample	Thiamin (mg/100g)	Recovery[2] (%)
Bio-Rex				
Room	40.4[a]	14.8	0.073	99.4±2.7[a]
Hot	41.2[a]	15.3	0.074	101.1±0.6[a]
Decalso				
Room	36.9[b]	14.7	0.080	90.1±1.5[b]
Hot	40.7[a]	16.4	0.080	99.4±3.8[a]
Significance of F-value[3]				
Resin	**	ns	**	**
Temperature	**	*	ns	**
Resin X Temp.	*	ns	ns	*
LSD[4]	1.67	-	-	4.42

[1] Means of 4 replications

[2] Based on fluorescence of the thiamin standard before adsorption on ion exchangers

[3] * significant at the 5% level; ** significant at the 1% level; ns not significant

[4] LSD, least significant difference at the 5% level

[a,b,c] Means with the same letter indicate no significant difference between those means

was based on comparison of the concentration of the thiamin standard with that of the sample extract. With the hot eluent, recovery was 99.4%. With the cold eluent (room temperature), recovery was 90.1% which is comparable to our previous recoveries of 93.9% (Table 1) under similar conditions. Therefore, room temperature elution recovered only 91% as much thiamin as hot temperature. Increasing eluent temperature increased the standard deviation from 1.5 to 3.8. Pippen and Potter (1975) reported that with cold eluent, mean recovery was only 77.2% and with hot eluent still only 88.4%, both of which were low compared with our results (90.1 and 99.4%). They may not have regulated eluent flow rate which can affect the thiamin recovery as we mentioned previously. Hence, not only eluent flow rate as mentioned in most publications on the thiochrome method, but also eluent temperature affected recovery of thiamin standard from Decalso with hot eluent recovering 9% more thiamin than cold eluent. Ellefson et al. (1981) reported similar recovery values to ours from Bio-Rex 70 and Decalso columns using hot eluent temperature. Their recovery of thiamin from Bio-Rex 70 (hydrogen form) was 95-100% and from Decalso was 90-100%.

Eluent volume. Results of the use of two different eluent volumes on two different cation exchangers for the purification step are reported in Table 3. Increasing eluent volume from 25 to 50 ml did not significantly increase fluorescence of thiamin standard solutions or thiamin recovery values from

either Bio-Rex 70 or Decalso columns. But there was a significant increase in the fluorescence of the sample extract ($p < 0.05$) when 50 ml was used.

The use of Bio-Rex 70 produced higher fluorescence of the thiamin standard and percentage thiamin recovery ($P < 0.001$), compared with those from Decalso. Pippen and Potter (1975) reported that increasing eluent volume from 25 to 50 ml for a Decalso column significantly increased thiamin recovery from 90 to 97.5% and decreased standard deviation from 2.15 to 1.53, indicating that eluent volume was a more important factor affecting thiamin recovery than eluent temperature. However, determined thiamin content values for fried chicken meat were less when Bio-Rex 70 was used than when Decalso was used and for 25 ml eluent than for 50 ml.

Generally, the use of Bio-Rex 70 resulted in higher and more consistant recovery of standard thiamin and less standard deviation than those from Decalso. Even though slightly lower determined thiamin values of chicken meat were obtained when Bio-Rex 70 was used, the thiamin recovery from Bio-Rex 70 was affected minimally by eluent flow rate, eluent temperature and eluent volume. Therefore, in the purification step for the thiamin determination, the use of Bio-Rex 70 can be recommended in place of Decalso.

Table 3-Instrument readings of fluorescence for thiamin standard and sample extract from ion exchange columns, thiamin content of fried chicken breast muscle and recovery of thiamin standard eluted with 25 ml or 50 ml of acid 25% KCl[1]

Volume	Fluorescence		Thiamin (mg/100g)	Recovery[2] (%)
	Standard	Sample		
Bio-Rex				
25 ml	42.4	27.5	0.087	97.7±1.4
50 ml	42.0	28.7	0.091	97.0±3.9
Decalso				
25 ml	39.2	27.3	0.092	90.6±3.0
50 ml	40.3	29.9	0.095	93.2±3.0
Significance of F-value[3]				
Resin	***	ns	*	***
Volume	ns	*	*	ns
Resin X Vol.	ns	ns	ns	ns
LSD[4]	-	-	-	-

[1] Means of 4 replications

[2] Based on fluorescence of the thiamim standard before adsorption on ion exchangers

[3] * significant at the 5% level; ** significant at the 1% level; *** significant at the 0.1% level; ns not significant

[4] LSD, least significant difference at the 5% level

a,b,c Means with the same letter indicate no significant difference between those means

Thiamin Content of Fried Chicken Muscles Cooked by Two Methods

Fat, moisture and thiamin contents of fried chicken breast muscle are presented in Table 4.

Fat. No significant difference was noted in percentage fat between chicken meat cooked by the two methods. Contrary results were reported by Mostert and Stadelman (1964), who found a greater percent ether extract of deep-fat fried broiler meat with zero pressure (12.2%) than that of deep-fat fried with 30 lbs pressure (10.9%). Bowers et al. (1985) found that percentage fat of open-vat fried chicken light meat including breading (19.5 - 20%) was greater than that of pressure fried (15.8%), probably due to the differences in fat absorption of coating. The lower fat content of our samples, either "Original" or "Crispy", was likely due to removal of coating which contains most fat absorbed from cooking medium.

Moisture. There were significant differences in percent moisture between samples cooked by the two methods ($p < 0.05$) with higher moisture for open-vat fried chicken breast muscle than for pressure fried. Conversely, Mostert and Stadelman (1964) obtained a lower percent moisture for deep-fat fried chicken meat with zero pressure (56.2%) than for that deep-fat fried with 30 lbs pressure (60.0%). Bowers et al. (1985) also obtained less moisture content from open-vat fried chicken meat (39.5-40.5%) than from those from pressure fried (47%). Fat

usually varies inversely with moisture. However, similar percentages of moisture for open vat fried chicken breast meat (33.1%) and pressure fried (32.1%) were reported in another publication (Kentucky Fried Chicken Corp., 1981). Compared with the results of previous studies, our higher percentage moisture in both "Original" and "Crispy" chicken was likely due to the removal of coating, which contains most of the fat and where evaporation of moisture mainly occured during the frying. Previous researchers mentioned that breading may function as a moisture barrier regardless of cooking methods (Love and Goodbwin, 1974; Proctor and Cunningham, 1983).

Thiamin. Thiamin was calculated on a wet - weight basis and also on a moisture and fat-free basis. On either basis, no difference was noted between thiamin contents of pressure and open-vat fried chicken breast muscles. Similar results were obtained by Bowers et al. (1985) who analyzed nutrient composition of commercially fried chicken coated with batter. Thiamin content of open-vat fried chicken was 0.19-0.23 mg/100g and for pressure fried 0.19 mg/100g. However, the thiamin content of open-vat fried chicken meat (0.12 mg on wet basis) was reported as twice as much as that of pressure fried in a publication of nutrient facts (Kentucky Fried Chicken, 1981). The comparision of thiamin content determined in our study with those reported in previous publications may be of little importance because sample preparation and methodology for

Table 4-Fat, moisture and thiamin content of Kentucky Fried Chicken breast muscle[1]

	Fat (%)	Moisture (%)	Thiamin	
			Cooked meat (mg/100g)	Moisture-fat free meat (mg/100g)
Original	2.20	65.9	0.066±0.014	0.207±0.042
Crispy	1.83	68.7	0.071±0.006	0.239±0.019
Significance of F-value[2]				
Type	ns	*	ns	ns
LSD[3]	-	1.34	-	-

[1] means of 8 replications

[2] * significant at the 5% level; ns not significant

[3] LSD, least significant difference at the 5% level

thiamin determination may influence results. Mickelsen et al. (1939) found that frying without pressure produced the least change in thiamin with fried beef round and fried pork ham. Pudelkewicz et al. (1963) reported that pressure cooking of chicken meat significantly reduced thiamin retention levels to 38%. Cover and Dilsaver (1947) also obtained very low thiamin retention (29%) in pressure-cooked lamb stews.

CONCLUSIONS

Based on the conditions of this study, we concluded:

1) Eluent flow rate and eluent temperature used in the purification step with Decalso significantly affected recovery of a standard thiamin solution: a slow flow rate (less than 1 ml per min) produced satisfactory recovery of more than 94%; hot eluent significantly increased thiamin recovery from 90.1 to 99.4%; eluent volume did not affect recovery.
2) Thiamin recovery from Bio-Rex 70 column was affected minimally by eluent flow rate, eluent temperature and eluent volume.
3) The use of Bio-Rex 70 for purification resulted in slightly lower thiamin value for chicken meat and higher and more consistent recoveries of standard thiamin solutions than when Decalso was used.
4) Thiamin contents of pressure fried chicken breast muscle and open-vat fried muscles were not significantly different.

REFERENCES

Ang, C.Y.W., Basillo, L.A., Cato, B.A., and Livingston, G.E. 1978. Riboflavin and thiamin retention in frozen beef-soy patties and frozen fried chicken heated by methods used in food service operations. J. Food Sci. 43: 1024.

AOAC. 1980 and 1984. "Official Methods of Analysis," 13th ed., Association of Official Analytical Chemists, Arlington, VA.

Bechtel, W.G. and Hollenbeck, C.M. 1958. A revised thiochrome procedure for the determination of thiamin in cereal products. Cereal Chem. 35: 1.

Bio-Rad Laboratories. 1982. Bulletin 1113.

Borenstein, B. 1975. Stability of nutrients in foods. Ch. 4. In "Nutritional Evaluation of Food Processing," 2nd ed., P. 16-17. (Ed.) Harris, R.S. and Karmas, E. AVI Publishing Co., Westport, CT.

Bowers, J.A. and Fryer, B.A. 1972. Thiamin and riboflavin in cooked and frozen, reheated turkey (gas vs. microwave ovens). J. Am. Dietet. A. 60: 399.

Bowers, J.A., Craig, J.A., Tucker, T.J., Holden, J.M., and Posati, L.P. 1985. Unpublished manuscript, "Vitamin and proximate composition of fast-food fried chicken." Dept. of Foods and Nutr., Kansas State Univ., Manhattan, KS.

Conner, R.T. and Straub, G.J. 1941. Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem., Anal. Ed. 13: 380.

Cover, S. and Dilsaver, E.M. 1947. Retention of the B vitamins in beef and lamb after stewing. 2. Thiamin. J. Am. Dietet. A. 23: 613.

Deutsch, M.J., Stephen Schiaffino, S., and Pillsbury, H.C. 1960. Stability and determination of thiamin in a cornmeal enrichment mixture. J.A.O.A.C. 43(1): 45.

Dwivedi, B.K. and Arnold, R.G. 1973. Chemistry of thiamin degradation in food products and model systems : A review. J. Agr. Food Chem. 21(1): 54.

Edijala, J.K. 1979. Modified fluorimetric procedure for the simultaneous determination of thiamin and riboflavin in cowpea. Analyst. 104: 637.

Ellefson, W.C., Richter, E., Adams, M., and Baillies, T.N.

1981. Evaluation of ion exchange resins and various enzymes in thiamin analysis. J.A.O.A.C. 64: 1336.

Feaster, J.F., Tompkins, M.D., and Ives, M. 1947. Influence of processing technique on vitamins in canned corn. Canner. 104(11): 16. [In Food Technol. (1957) 11: 77]

Feliciotti, E. and Esselen, W.B. 1957. Thermal destruction rates of thiamin in pureed meats and vegetables. Food Technol. 11: 77.

Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497.

Freed, M. 1966. "Methods of Vitamin Assay," 3rd ed., p. 129. Interscience, New York, NY.

Gilbert, L., Bowers, J.A., Craig, J.A., and Cunningham, F.E. 1981. Sensory characteristics and thiamin and vitamin B_6 contents of spent hens. Poultry Sci. 60: 1079.

Guthrie, H.A. 1983. Basic principles of nutrition. Part 1. In "Introductory Nutrition," 5th ed., p.286. The C.V. Mosby Co., St. Louis, Missouri.

Hall, K.N. and Lin, C.S. 1981. Effect of cooking rates in electric or microwave oven on cooking losses and retention of thiamin in broilers. J. Food Sci. 46: 1292.

Halliday, N. and Deuel, H.J. 1941. The presence of free and combined thiamin in milk. J. Biol. Chem. 140: 555.

Hampel, C.A. and Hawley, G.G. (Ed.) 1973. "The Encyclopedia of Chemistry," 3rd ed. Van Nostrand Reinhold Co., New York, NY.

Harris, L.J. and Wang, Y.L. 1941. An improved procedure for estimating vitamin B_1 in foodstuffs and biological materials by the thiochrome test including comparisons with biological assays. Biochem. J. 33: 1050.

Hennessy, D.J. and Cerecedo, L.R. 1939. The determination of free and phosphorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. 61: 179.

Hennessy, D.J. 1941. Chemical methods for determination of vitamin B_1. Ind. Eng. Chem., Anal. Ed. 13: 216.

Hinman, W.F., Halliday, E.G., and Brookers, M.H. 1944. Thiamin in beef muscles. Ind. Eng. Chem., Anal. Ed. 16: 116.

Jackson, S.H., Crook, A., Malone, V., and Drake, T.G.H. 1945. The retention of thiamin, riboflavin and niacin in cooking pork and in processing bacon. J. Nutr. 29: 391.

Jansen, B.C.P. 1936. A chemical determination of aneurin by the thiochrome reaction. Rec. Trav. Chim. 55: 1046.

Jowett, M. 1940. The estimation of vitamin B_1 in urine. Biochem. J. 34: 1348.

Kentucky Fried Chicken Corp. 1981. Nutritional facts about Kentucky Fried Chicken.

Kline, O.L. 1944. Report on vitamin B_1. J.A.O.A.C. 27: 534.

Lachance, P.A., Ranadive, A.S., and Matas, J. 1973. Effects of reheating convenience foods. Food Technol. 27(1): 36.

Love, F.E. and Goodwin, T.L. 1974. Effects of cooking methods and browning temperatures on yields of poultry parts. Poultry Sci. 53: 1391.

Lund, D.B. 1973. Effects of heat processing. Food Technol. 27: 16.

MacBride, D.E. and Wyatt, C.J. 1983. Evaluation of a modified AOAC determination for thiamin and riboflavin in foods. J. Food Sci. 48: 748.

Maier, G.D and Metzler, D.E. 1957. Structures of thiamin in basic solution. J. Amer. Chem. Soc. 79: 4386.

McRoberts, L.H. 1960. Determination of thiamin in enriched cereal and bakery products. J.A.O.A.C. 43: 47.

Melnick, D. and Field, H. 1939. Chemical determination of vitamin B_1. 3. Quantitative enzymic conversion of cocaboxylase (Thiamin pyrophosphate) to the free vitamin. J. Biol. Chem. 127: 531.

Mickelsen, O., Waisman, H.A., and Evelhjem, C.A. 1939. The distribution of vitamin B_1 (Thiamin) in meat and meat products. J. Nutr. 17: 269.

Morgan, B.H. 1971. Edible packaging update. Food Product Dev. 5: 75.

Mostert, G.C. and Stadelman, W.J. 1964. Effect of method of cookery on shrinkage, moisture and ether extractable content of broiler legs and thighs. Poultry Sci. 43: 896.

Pelletier, O. and Madere, R. 1972. New automated method for measuring thiamin (Vitamin B_1) in urine. Clin. Chem. 18: 937.

Pelletier, O. and Madere, R. 1975. Comparison of automated and manual procedures for determining thiamin and riboflavin in foods. J. Food Sci. 40: 374.

Pike, R.L. and Brown, M.L. 1984. The nutrients. Part 1. In "Nutrition; An Integrated Approach," 3rd ed., p.88-89. John Wiley and Sons, Inc., New York.

Pippen, E.L. and Potter, A.L. 1975. Quantitating the recovery of thiamin (vitamin B_1) from Decalso in the thiochrome method for the determination of thiamin. J. Agric. Food Chem. 23: 523.

Proctor, V.A. and Cunningham, F.E. 1983. Composition of broiler meat as influenced by cooking methods and coating. J. Food Sci. 48: 1696.

Pudelkewicz, C., Gordon, H., Whitworth, L., Caldwell, H.M., and Kahlenberg, O.J. 1963. Effects of processing and cooking on certain nutrients in fowl. Agri. Exp. Station Res. Bull. No. 819, University of Missouri, Columbia, MO.

Rice, E.E. and Beuk, J.F. 1945. Reaction rates for decomposition of thiamin in pork at various cooking temperatures. Food Res. 10: 99.

Rindi, G. and deGiuseppe, L. 1961. The content of thiamin and its mono-, di- and tri-phosphoric esters in different biological materials. Intern. Z. Vitaminforsch 31: 321.

Strohecker, R. and Henning, H.M. 1966. "Vitamin Assay: Tested Methods," translated by Libman, D.D. Chemical Rubber Publishing Co., Cleveland, Ohio, 2nd Printing, revised. p. 65-97.

Tucker, R.E., Hinman, W.F., and Halliday, E.G. 1946. The retention of thiamin and riboflavin in beef cuts during braising, frying, and broiling. J. Am. Dietet. A. 22: 877.

APPENDIX

APPENDICES

1. Modification and specifications of AOAC (1980) thiochrome method for thiamin assay
2. Fat determination (modification of Folch method)
3. Tables (5-15) and Figures (2-4)

MODIFICATION AND SPECIFICATIONS OF AOAC (1980) THIOCHROME METHOD FOR THIAMIN ASSAY

Reagents and apparatus

Decalso (30 - 80 mesh, Thiocrome Decalso, Permutit T, Fisher Scientific Co. No. T-97) was reactivated after one cycle of thiamin adsorption and elution with acid-KCl, as described by Freed (1966). Bio-Rex 70 (50 - 100 mesh, sodium form, purchased from Bio-Rad Laboratories, Richmond, CA) was converted to hydrogen form as in Ellefson et al. (1981). The glass chromatographic tubes (22.5 cm overall length, with a reservoir capacity of 30 ml) having capillary tubes (11 cm long x 6 mm o.d.) were used. Polyethylene tubes (2.5 mm o.d. x 9 cm long) with plastic clamps were attached to the tip of capillary to control the four different flow rates (1/2, 1, 1 1/2 and 2 ml per min approximately).

Extraction

Ten g of sample were weighed into a 100 ml volumetric flask; 75 ml of 0.1N HCl added and sample autoclaved for 15 min at 15 lb pressure.

Enzyme hydrolysis

A fresh 6% solution of Diastase (D-21 obtained from Fisher Scientific Co. Fair Lawn, New Jersey 07410) was prepared in 2.5M sodium acetate. Five ml of enzyme suspension was added to the sample extract with final pH adjusted to 4.5-5.0

followed by incubation in a Model No. 702A incubator (Labline, Inc.) for 16hr at 45-50°C.

Purification

Twenty-five ml of the filtrate of sample extract or standard thiamin solution was allowed to run into the Decalso or Bio-Rex 70 column followed by three 10 ml hot water washes. Then, 20 ml of acid KCl or second 20 ml portion was placed in the reservoir, collecting the eluate in either a 25 ml or a 50 ml volumetric flask and diluting the contents to the mark with acid KCl solution. For cold elution, acid KCl reagent was at room temperature. For hot elution, the reagent was heated to 90-95°C on the Corning Hot Plate (Model PC-101). The conditions of three experiments designed to study flow rate, eluent temperature and eluent volume using two different resin, were regulated as follows: (1)For flow rate variation, 25 ml of eluent at room temperature was used; (2)for eluent temperature variation, 1 1/2 ml per min of flow rate for Bio-Rex 70 column and 1 ml per min for Decalso and 25 ml of eluent volume were used; and (3)for eluent volume variation, 1 1/2 ml per min of flow rate for Bio-Rex 70 column and 1 ml per min for Decalso and room temperature of eluent were used.

Oxidation of thiamin to thiochrome

Three ml of alkaline ferricyanide solution were added slowly to 5 ml of the acid KCl eluent of sample or thiamin standard

and swirled for about 2 sec. Then, 15 ml of isobutyl alcohol was added immediatly with a repipet, shaken vigorously for 90 sec on Wrist Action Shaker (Burrell Corp. Pittsburgh, PA. Model No. 75), centrifuged briefly to speed phase separation, and the aqueous layer (lower) siphoned out. Approximately 0.25 teaspoon of anhydrous sodium sulfate, used to dry the remaining isobutyl alcohol layer, was added to each reaction vessel and allowed to stand until sparkling clear.

Preparation of blank

One to two drops of concentrated hydrochloric acid were added to the cuvet containing the isobutyl alcohol solution of thiochrome whose fluorescence had been measured and mixed well and fluorescence read with a Coleman model 12C fluorometer by inserting a primary filter (B-1) between the light source and the sample tube and a secondary filter (PC-1) between the sample tube and the photocell.

FAT DETERMINATION (MODIFICATION OF FOLCH METHOD)

(1) Weigh 5 g of sample in 50 ml centrifuge tube.

(2) Add 30 ml extraction fluid (methylene chloride:methyl alcohol = 2:1) and blend with the Polytron (Ser. No. 10210) for 30 sec.

(3) Shake on a Wrist Action Shaker for 5 min.

(4) Centrifuge for 5 min.

(5) Decant liquid through filter paper (Whatman No. 1) and collect in clear centrifuge tubes.

(6) Reextract with 15 ml of extraction fluid and shake for 5 min on Wrist Action Shaker.

(7) Filter into clear centrifuge tube.

(8) Add 8 ml of 0.73% NaCl to each set of centrifuge tubes from (5) and (7) and shake the tubes for 2 min.

(9) Centrifuge for 2 min.

(10) Aspirate the aqueous layer (top) with a pasteur pipette attatched to a water pump.

(11) Pour the remaining solvent layer into preweighed aluminum pan. Both extractions are placed into the same pan for evaporation.

(12) Place the pans in a drying oven at 105-110^{o}C for 1 hr.

(13) After drying cool in a desiccator for 30 min and weigh.

Recovery (%) of thiamin standard from ion-exchange columns was based on the average of 2 readings of fluorescence for 2 duplicate thiamin standards before adsorption on ion exchangers.

Table 5-Instrument readings of fluorescence for thiamin standard and sample extract from ion-exchange columns at various flow rates (ml/min)

Flow Rate	Replication 1	2	3	4	Mean
Standard					
Bio-Rex					
1/2	37.2	38.5	38.0	40.0	38.4
1	39.0	37.0	38.0	40.0	38.5
1 1/2	39.5	39.0	36.8	39.5	38.7
2	38.8	36.5	38.8	40.0	38.5
Decalso					
1/2	38.5	39.3	36.5	38.5	38.2
1	41.9	34.3	38.5	33.8	37.1
1 1/2	32.6	31.0	32.3	34.6	32.6
2	28.8	31.8	33.3	36.7	32.7
Sample					
Bio-Rex					
1/2	18.0	12.0	18.0	17.5	16.4
1	17.3	12.0	18.0	16.0	15.8
1 1/2	18.5	11.3	16.5	15.5	15.5
2	16.0	11.0	16.3	13.5	14.2
Decalso					
1/2	17.8	12.0	16.3	17.0	15.8
1	16.9	13.0	17.5	15.8	15.8
1 1/2	16.5	10.0	16.0	13.4	14.0
2	13.5	12.5	14.8	13.3	13.5

Table 6-Recovery (%) of thiamin standard from ion-exchange columns at various flow rates (ml/min)

	Replication				
Flow Rate	1	2	3	4	Mean
Standard					
Bio-Rex					
1/2	93.0	96.7	100.0	98.8	97.1±3.1
1	97.5	92.5	100.0	98.8	97.2±3.3
1 1/2	98.8	97.5	96.8	97.5	97.7±0.8
2	97.0	91.3	102.0	98.8	97.3±4.5
Decalso					
1/2	96.3	98.3	96.1	95.1	96.5±1.3
1	104.8	85.8	101.3	83.5	93.9±10.8
1 1/2	81.5	77.5	85.5	85.4	82.5±3.8
2	72.0	79.5	87.6	90.6	82.4±8.3

Table 7-Thiamin content (mg/100g) of fried chicken breast muscle purified from ion-exchange columns at various flow rates (ml/min)

	Replication				
Flow Rate	1	2	3	4	Mean
Bio-Rex					
1/2	0.097	0.062	0.095	0.088	0.085
1	0.088	0.065	0.095	0.080	0.082
1 1/2	0.094	0.058	0.090	0.079	0.080
2	0.087	0.060	0.084	0.068	0.075
Decalso					
1/2	0.090	0.081	0.100	0.093	0.082
1	0.062	0.076	0.065	0.079	0.086
1 1/2	0.089	0.091	0.098	0.089	0.085
2	0.088	0.094	0.078	0.073	0.084

Table 8-Instrument readings of fluorescence for thiamin standard and sample extract from ion-exchange columns at room and hot temperature (90-95°C)

Tempe-rature	Replication 1	2	3	4	Mean
Standard					
Bio-Rex					
Room	39.8	38.8	43.3	39.8	40.4
Hot	39.5	39.0	44.0	42.3	41.2
Decalso					
Room	35.3	34.8	40.3	37.0	36.9
Hot	38.0	37.8	43.5	43.5	40.7
Sample					
Bio-Rex					
Room	13.0	14.5	15.0	16.5	14.8
Hot	13.0	14.8	16.5	16.8	15.3
Decalso					
Room	13.5	12.5	15.9	16.7	14.7
Hot	14.0	15.0	16.5	20.0	16.4

Table 9-Recovery (%) of thiamin standard from ion-exchange columns at room and hot temperature (90-95°C)

Temperature	Replication 1	2	3	4	Mean
Bio-Rex					
Room	102.1	100.8	98.5	95.9	99.4±2.7
Hot	101.3	100.8	100.5	101.9	101.1±0.6
Decalso					
Room	90.5	88.5	92.0	89.2	90.1±1.5
Hot	97.4	96.2	99.3	104.8	99.4±3.8

Table 10-Thiamin content (mg/100g) of fried chicken breast muscle purified from ion-exchange columns at room and hot temperature (90-95°C)

Tempe-rature	Replication 1	2	3	4	Mean
Bio-Rex					
Room	0.065	0.075	0.069	0.083	0.073
Hot	0.066	0.075	0.075	0.079	0.074
Decalso					
Room	0.077	0.072	0.075	0.079	0.074
Hot	0.074	0.079	0.076	0.092	0.080

Table 11-Instrument readings of fluorescence for thiamin standard and sample extract from ion-exchange columns eluted with 25 or 50 ml of acid KCl

	Replication				
Volume	1	2	3	4	Mean
Standard					
Bio-Rex					
25 ml	42.0	42.8	41.8	42.3	42.2
50 ml	40.8	42.6	40.8	43.6	42.0
Dcalso					
25 ml	38.0	39.8	38.5	40.3	39.2
50 ml	40.0	40.8	40.8	39.6	40.3
Sample					
Bio-Rex					
25 ml	28.5	29.3	26.8	25.3	27.5
50 ml	29.4	28.8	28.6	28.0	28.7
Decalso					
25 ml	29.5	27.8	27.9	23.8	27.3
50 ml	34.0	29.8	31.0	24.6	29.9

Table 12-Recovery (%) of thiamin standard from ion-exchange columns eluted with 25 or 50ml of acid KCl

	Replication				
Volume	1	2	3	4	Mean
Bio-Rex					
25 ml	97.7	97.3	96.1	99.5	97.7±1.4
50 ml	94.9	96.8	93.8	102.6	97.0±3.9
Decalso					
25 ml	88.4	90.5	88.5	94.8	90.6±3.0
50 ml	93.0	92.7	93.8	93.2	93.2±0.5

Table 13-Thiamin content (mg/100g) of fried chicken breast muscle purified from ion-exchange columns eluted with 25 or 50 ml of acid KCl

	Replication				
Volume	1	2	3	4	Mean
Bio-Rex					
25 ml	0.090	0.091	0.085	0.080	0.087
50 ml	0.096	0.090	0.093	0.086	0.091
Decalso					
25 ml	0.100	0.090	0.097	0.079	0.092
50 ml	0.100	0.097	0.101	0.083	0.095

Table 14-Fat and moisture content of fried chicken breast muscle

Replication	Fat, %		Moisture, %	
	Original	Crispy	Original	Crispy
1	2.50	1.97	64.2	69.3
2	2.16	1.64	66.5	69.2
3	2.51	1.85	65.5	69.1
4	1.73	1.46	67.1	69.1
5	2.74	2.47	64.0	65.7
6	1.75	1.61	67.0	69.0
7	2.31	2.02	66.5	68.2
8	1.87	1.65	66.4	69.8
Mean	2.20	1.83	65.9	68.7

Table 15-Thiamin content of fried chicken breast muscle

Relication	Thiamin, mg/100g			
	Wet weight basis		Moisture and fat-free weight basis	
	Original	Crispy	Original	Crispy
1	0.075	0.064	0.225	0.223
2	0.046	0.075	0.147	0.257
3	0.053	0.066	0.166	0.227
4	0.075	0.080	0.241	0.272
5	0.076	0.072	0.229	0.226
6	0.080	0.068	0.256	0.231
7	0.072	0.066	0.231	0.222
8	0.051	0.073	0.161	0.256
Mean	0.066	0.071	0.207	0.239

Fig. 2-Effect of eluent flow rate on mean recovery (%) of thiamin standard

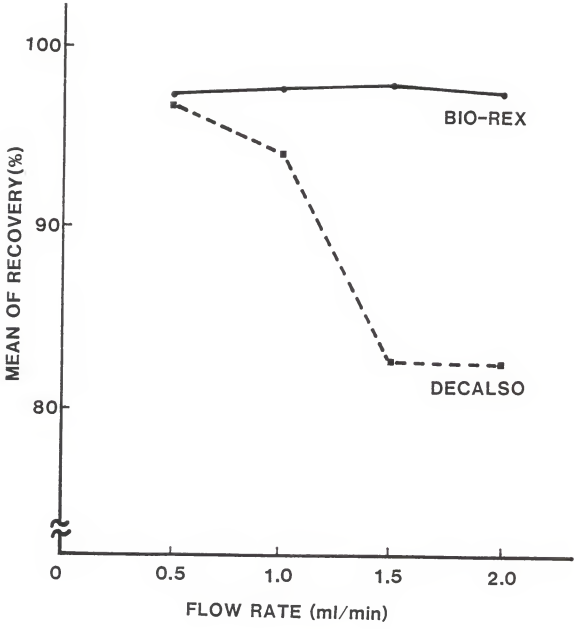

100
90
80
MEAN OF RECOVERY(%)
BIO-REX
DECALSO
0
0.5
1.0
1.5
2.0
FLOW RATE (ml/min)

Fig. 3-Effect of eluent temperature on mean recovery (%) of thiamin standard

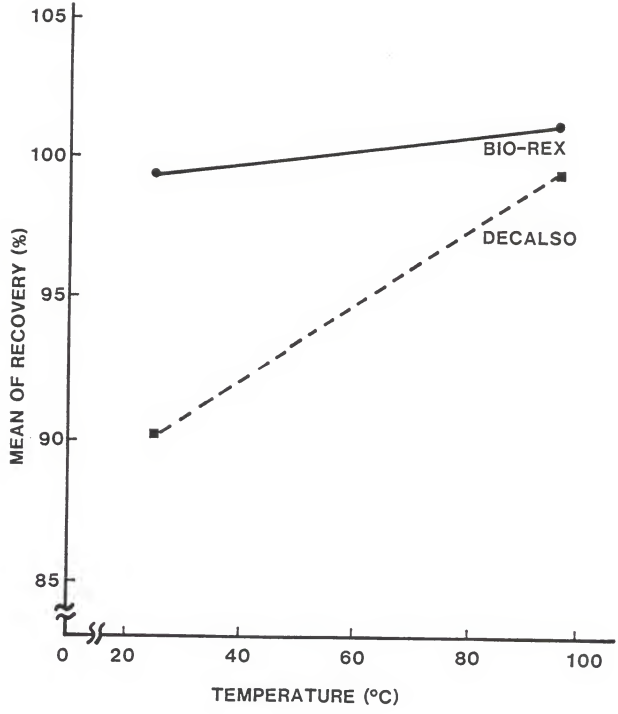
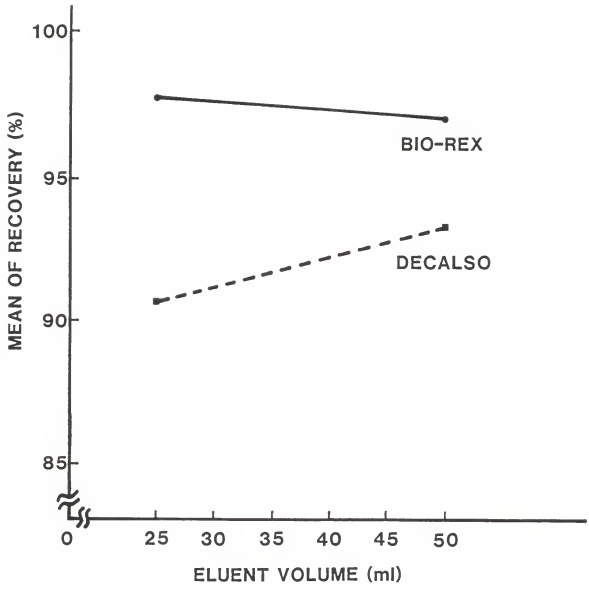

105
100
95
90
85
MEAN OF RECOVERY (%)
BIO-REX
DECALSO
0
20
40
60
80
100
TEMPERATURE (°C)

Fig. 4-Effect of eluent volume on mean recovery (%) of thiamin standard


100
95
90
85
MEAN OF RECOVERY (%)
BIO-REX
DECALSO
0
25
30
35
40
45
50
ELUENT VOLUME (ml)

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MODIFICATION OF FLUOROMETRIC ASSAY FOR THIAMIN IN CHICKEN MUSCLE

by

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B. S., Seoul National University, 1975

AN ABSTRACT OF A MASTER'S THESIS

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Effects of selected variables (flow rate, temperature and volume of eluent using two resins, Decalso and Bio-Rex 70) on the determined thiamin values of fried chicken meat and the effect of cooking methods on thiamin content of fast-food fried chicken breast muscle using a modified AOAC method were investigated. Four eluent flow rates (1/2, 1, 1 1/2 and 2 ml per min), two temperatures (hot and room) and two volumes (25 and 50 ml) were used during the purification of sample extracts. Thiamin contents of pressure fried chicken breast muscle and open-vat fried were determined using Bio-Rex 70 column with a flow rate of 1 1/2 ml per min and 25 ml of eluent volume at room temperature.

Eluent flow rate ($p < 0.05$) and temperature ($p < 0.01$) used in purification step with Decalso significantly affected recovery (%) of standard thiamin but eluent volume did not: slow flow rate (less than 1 ml per min) produced satisfactory recovery of more than 94% and the use of a fast flow rate (more than 1 1/2 ml per min) resulted in a low recovery value (around 82%); hot eluent temperature significantly increased thiamin recovery from 90.1 to 99.4%. Thiamin recovery from Bio-Rex 70 was affected minimally by eluent flow rate, eluent temperature and eluent volume; a recovery value of about 97% was obtained from Bio-Rex 70 at all flow rates with the highest value at 1 1/2 ml per min. Generally, the use of Bio-Rex 70 resulted in lower thiamin values for fried chicken meat and higher and more consistant recovery of a standard thiamin

solution than when Decalso was used.

Percentages of fat for pressure fried chicken breast muscle and open-vat fried were not significantly different, but percentage moisture of open-vat fried chicken was significantly higher than for pressure fried chicken ($p < 0.05$). The thiamin contents of chicken breast muscles cooked by the two methods were not significantly different (pressure fried chicken, 0.207; open-vat fried chicken, 0.239 mg/100g on a moisture and fat-free basis).